Use of an analysis substance for detecting an explosive.

Field of the invention.

The invention relates to a use of an analysis substance for detecting an explosive, wherein the analysis substance specifically binds to at least one partial molecular structure of the explosive and wherein a binding event between the partial molecular structure and the analysis substance is detected, and to analysis substances for such uses or methods.

Such analysis substances can be used for instance in analytical investigations of the environment, in particular of the air, drinking water and/or soil, however also in other sectors of biochemical analytical and medical diagnostic investigations for detecting explosives or their main chemical components. Further, the application is also possible for security measures, for instance for the presence of explosives in transportation goods, such as flight baggage and the like.

Explosives in the meaning of DIN 20163 (Sept. 1985) are such explosive substances, which are technically used as explosives, driving substances or gun powders, ignition agents, initiating substances or pyrotechnic products. Exam-

ples for explosives are organic nitro compounds such as TNT (trinitrotoluene), nitramine (hexogen=RDX=hexahydro-1,3,5-trinitro-1,3,5-triazine), nitrosamine and picric acid. However, inorganic substances are also to be named here, such as lead azide.

Analysis substances are substances, which are used in an analysis method for determining the type and amount of a substance, wherein the amount of bound analysis substance is determined directly or indirectly semi-quantitatively or quantitatively, and therefrom the amount or concentration of an explosive is determined and displayed. The term semi-quantitative determination also comprises an information with regard to exceeding or falling below a defined limit amount of bound analysis substance and consequently of a limit amount or limit concentration of explosive correlated herewith (present/absent in the case of a limit amount, which is determined by the detection sensitivity).

A binding event may be any kind of a (physical-)chemical binding/interaction, e.g. ionic binding, covalent binding, van der Waals forces or hydrogen bridge bindings.

A partial molecular structure may be a functional group, a combination of several functional groups, in particular of adjacent groups, or also a carbon structure with or without functional groups. It is characteristic that it is a section of a molecule or a compound and not the whole molecule. Corresponding considerations apply in the case of inorganic explosive molecules.

The detection of a binding event may be made by optical, chemical, biological or other physical or physical-technical methods.

Background of the invention and prior art.

Explosives can on one hand be a substantial risk because of the explosive properties. For instance in security areas, such as airports etc, explosives have to be detected, for instance in order to prevent undesired actions under utilization of the explosives by persons. On the other hand, explosives are further in most cases human toxic and/or ecotoxic, and it is therefore desirable to be able to detect even smallest amounts in soil or water samples, also in aerosols, preferably including the detection of the specific explosive found. The latter is of special importance for instance for conversion measures of abandoned military facilities. Finally, for forensic purposes, it is often necessary to detect explosive traces and to identify the type of explosive.

In the practice, different classic (wet-) chemical analysis methods are known in the art. Their application requires quite some efforts, they need a lab (on-site measurements are normally not possible) and do not supply quick results. Further, the achievable detection limits are not satisfying. Samples have before to be concentrated-up in an expensive way, in order to fall below the high detection limit of these test systems. Such concentrating-up of samples is further time-consuming and cost-intensive. Moreover, the in so far known test systems show

cross sensitivities to further substances included in the samples.

A fiber-optic biosensor for detecting TNT based on an immunoassay using a fluorescent detector compound, namely antibodies, is known from the document Craig, H. et al., Field Demonstration of On-Site Analytical Methods for TNT and RDX in Ground Water, Proceedings of the HSRC/WERC Joint Conference on the Environment, May 1996. Methods to be used therewith quantitatively detect explosives at most insufficiently; problematic is further the cross reactivity of antibodies with other substances.

Furthermore, it is known from the practice to use gas or liquid chromatography for detecting explosives. Measurement devices for this cannot be used on site.

In a sector of different technology, a biosensor using an immobilized fluorescence-marked aptamer for detecting thrombin is described (Potyrailo, RA. et al., Adapting Selected Nucleic Acid Ligands (Aptamers) to Biosensors, Analytical Chemistry, 70, 3419-3425, 1998). Another biosensor for detecting thrombin is disclosed in the document Lee, M. et al., A Fiber-Optic Microarray Biosensor Using Aptamers as Receptors, Analytical Biochemistry, 282, 142-146, 2000. This document describes the use of an aptamer immobilized on glass microballs for detecting thrombin.

Technical object of the invention.

It is the technical object of the invention to provide a method for detecting explosives, by means of which explosives can be detected with an improved sensitivity and specificity, and which permits the determination of explosives in the field, and to specify analysis substances therefor.

Basics of the invention.

For achieving this technical object, the invention teaches the use of a nucleic acid for detecting a synthetic explosive, wherein the nucleic acid specifically binds to a partial molecular structure or the overall molecular structure of the explosive, and wherein a binding event between the partial molecular structure or the overall molecular structure and the nucleic acid is detected.

A nucleic acid in the meaning of the invention may contain an RNA, DNA or a PNA as a nucleotide sequence, which may also comprise derivatized non-natural nucleotides. In addition to the nucleotide sequence, a nucleic acid may however also contain molecules, for instance bound at the terminal of the nucleotide sequence, which do not contain nucleotides. The nucleic acid may in particular be an aptamer. An aptamer is a nucleic acid, which comprises, analogously to an antibody/antigen affinity ("key/lock") or according to the binding model of the induced fit, a binding affinity to certain target structures on a molecular level. The oligonucleotide may also be a spiegelmer. A spiegelmer is a highly affinitive mirror image nucleic acid,

which is composed of L-ribose or L-2'-desoxyribose units.

It is achieved by the invention, compared to the classic analysis methods, that an extremely low detection limit for the detection of explosives is obtained, and thus the sensitivity of the test system for the detection of explosives is increased. An enrichment step performed before the measurement for concentrating-up the explosives is not necessary because of the high detection sensitivity and needs not to be made. Advantageous with regard to the antibody technology is (in addition to the better sensitivity and the on-site usability) that aptamers are completely in vitro identifiable (for instance by means of theoretical 3-dimensional structure calculations) and producible, and therefore no test animals are needed for immunization purposes. Nevertheless, a specificity is obtained, which is at least equivalent to those of the antibody technology, by far superior with regard to classic analytical methods.

The invention further teaches the sequences mentioned in the examples of execution for use in a method according to the invention.

The invention is based on the finding that nucleic acids can be found out based on their selectivity or affinity to a target molecule. Found nucleotide sequences can so to speak fold around a partial molecular structure, in particular in the case of small target molecules however also around an overall molecular structure, whilst other nucleotide sequences do not or to a smaller extent have this property and are rejected by a screening.

Detailed description of preferred embodiments.

The partial molecular structure of the explosive may carry available oxygen directly bound to a nitrogen atom and be selected from the group consisting of "nitrites, nitrates, nitro and nitroso compounds". The explosive may be selected from the group consisting of "nitrobenzol derivatives, TNT, 2,4-DNT, 2,6-DNT, 2-NT, picric acid, hexogen, octogen, hexyl, tetryl, ethylene glycol dinitrate, diethylene glycol dinitrate, nitroglycerin, nitropenta and derivatives of such compounds".

The nucleic acid may be selected from the group consisting of "sequences of Figs. 8 and 9 or any fragments of these sequences, as far as these fragments comprise at least 6, 10 or 15 consecutive nucleotides from such a sequence". Preferred are marked (underlined in Fig. 8) nucleic acids containing consensus sequences. The nucleic acid may be immobilized directly at a solid body surface, alternatively indirectly via a spacer compound at the solid body surface. A spacer compound is a connection molecule between a solid body surface and a nucleic acid or an aptamer. The spacer compound may be a linker nucleic acid, for instance a short synthetic DNA double strand; however, any other elongated organic molecules, typically oligomers or polymers, are also suitable. Further, a binding by usual affinity pairs, such as biotin/streptavidin, is also possible, a molecule of the affinity pair being connected with the nucleic acid, and the complementary molecule being immobilized. The solid body surface may be provided

as an optic fiber. It is understood that several different nucleic acid species may also be immobilized on the surface or fiber. In this case, different types of explosives each specifically binding to the respective nucleic acid species can simultaneously be detected.

A binding event can be detected by measurement of a signal of a detector molecule being (directly or indirectly) marked and competitively replaced in the binding to the nucleic acid by a molecule of the explosive. In particular, a fluorescence marking of the detector molecule can be used. A fluorescence marking is a marking with a fluorochrome. A fluorochrome may be for instance fluorescein, acridine orange or other usual fluorochromes such as Cy5, Cy3 etc. The signal can be generated by decrease of the signal intensity of bound detector molecules or by increase of the signal intensity of released (replaced) detector molecules. In the case of co-operative effects, such as stacking, an increase of the signal intensity of bound detector molecules can also take place, or for instance by FRET methods. In the case of the simultaneous use of different nucleic acid species, it may be recommendable to provide differently marked detector molecules for the respective nucleic acid species, in order that a discrimination between signals of different nucleic acid species is possible.

The invention further teaches a device for detecting an explosive, the device being equipped with a nucleic acid specific for a partial molecular structure of the explosive. The nucleic acid may be immobilized directly or alternatively by a spacer compound at a solid body

surface preferably of an optic fiber. It is preferred that the device comprises means for detecting a binding event between the partial molecular structure and the nucleic acid, for instance a fluorescence detector. In the device may be provided a light source for the fluorescence excitation of the detector molecules, the optic fiber being connected to a fluorescence detector. Furthermore, means for feeding a sample to the nucleic acid may be integrated. In the case of the detection of explosives in the security sector, for instance an air sample may be taken from the environment of objects to be monitored, and analyzed. Before the detection the air sample may first be brought into a liquid phase, where then a detection takes place as described. However, a detection in the gas phase is also possible, and for instance, nucleic acids and/or detector molecules used according to the invention may be contacted as an aerosol with the gas sample. The nucleic acid may be loaded with a fluorescence-marked detector molecule, and the binding force between the nucleic acid and the detector molecule should be smaller than the binding force between the nucleic acid and the partial molecular structure. A part of the optic fiber may be arranged in a sample gas or liquid space, whereinto a gas or liquid sample can be supplied. The wavelength of the irradiated light is preferably in the range of smaller wavelengths than the wavelength of the transmitted light of the marker. These may be wavelengths of the fluorescence range. The light may be introduced via an optic fiber, via the envelope surface thereof, but also via one or both front faces. The same applies to the exit of the transmitted light (fluorescence signal). The optic fiber may be rotatably supported. Gen-

erally, it is understood that transmitted light is not detected directly, but indirectly by emissions from molecules, which in turn are excited by the transmitted light. In this way, an optic amplification can also be achieved.

It is understood that the detection of the replacement of the marker may also take place in a different way, for instance by means of an electro-chemical sensor. Further, the concentration of the non-bound marker being directly proportional to the analyte amount may for instance be quantified for instance with an electro-enzymatic amplifier system. Thereby, increased sensitivities of the test system can be achieved.

A mobile measuring instrument based on fiber optics can be operated with a portable power supply, e.g. a battery. For recording and evaluating the measured signals, the measuring instrument may be provided with an electronic component, for instance a computer, and may comprise a transport device, for instance a hose pump, for transporting a gas or liquid sample.

In the following, the invention will be explained in more detail with reference to figures representing an example of execution only.

Fig. 1: adsorption of an exemplary explosive to an aptamer immobilized by a spacer compound.

Fig. 2: an aptamer immobilized by a spacer compound at an optic fiber, loaded with a fluorescence-marked detector molecule - before contact with a sample containing an ex-

plosive, in the dark without light irradiation.

Fig. 3: an aptamer immobilized by a spacer compound at an optic fiber, loaded with a fluorescence-marked detector molecule - before contact with a sample containing an explosive, light irradiation. The optic fiber conducts the light transmitted by the fluorochrome within the optic fiber.

Fig. 4: an aptamer immobilized by a spacer compound at an optic fiber, loaded with a fluorescence-marked detector molecule - after contact with a sample containing an explosive, in the dark without light irradiation. The explosive has replaced several detector molecules and is bound to the aptamer.

Fig. 5: an aptamer immobilized by a spacer compound at an optic fiber, loaded with a fluorescence-marked detector molecule - after contact with a sample containing an explosive, light irradiation. The explosive has replaced several detector molecules and is bound to the aptamer. The optic fiber conducts the light transmitted by the fluorochrome within the optic fiber. The emission is lower than in Fig. 2, since less fluorochromes were excited.

Fig. 6: a diagrammatic representation of a device according to the invention.

Fig. 7: comparative tests of the binding strength with an aptamer relative to an antibody in an immune method.

Fig. 8a to 8h: aptamer sequences according to the invention.

Fig. 9: consensus sequences according to the invention.

In Fig. 1 can be seen a nucleic acid 1 for detecting a synthetic explosive 2, the nucleic acid 1 specifically binding to a partial molecular structure 3 of the explosive 2. The explosive 2 is TNT. Suitable nucleic acids 1 are represented in Figs. 8 to 9.

Figs. 2 to 5 show the detection mechanism for explosives. The nucleic acid 1 is immobilized by a spacer compound 6 at a solid body surface 7, for instance the surface of an optic fiber 8. Fig. 2 represents the loading of the nucleic acid 1 with a fluorescence-marked 4 detector molecule 5, and Fig. 3 represents the detection of a binding event by measurement of a signal of a fluorescence-marked 4 detector molecule 5. The replacement of the detector molecule 5 from the binding with the nucleic acid 1 by a molecule of the explosive 2 is shown in Fig. 4. In Fig. 5 is shown that the signal is generated by a decrease of the signal intensity of bound detector molecules 5.

In Fig. 6 in combination with Figs. 1 and 5 can be seen a device for detecting an explosive 2 with a nucleic acid 1 being specific for a partial molecular structure 3 of the explosive 2. The nucleic acid is immobilized at a solid body surface 7. The nucleic acid 1 is immobilized by a spacer compound 6 at an optic fiber 8 and loaded with a fluorescence-marked 4 detector molecule 5. A light source 11 for the fluores-

cence excitation of the detector molecules 5 is provided, the optic fiber 8 being connected to a fluorescence detector 9 and a part of the optic fiber 8 being arranged in a sample gas or liquid space 12, whereinto a gas or liquid sample 13 can be supplied. A mobile measuring instrument based on fiber optics can be operated with a portable power source 14, e.g. a car battery. For recording and evaluating the measured values, the measuring instrument may be provided with an electronic component, for instance a computer 15, and may comprise a transport device 16, for instance a hose pump, for transporting the gas or liquid sample. For collecting the sample, a collector container 17 may be provided.

In Fig. 7, comparative tests of the binding strength are shown. There are shown binding investigations TNT/nucleic acid according to the invention compared to TNT/antibody. The dissociation constant of the aptamer reaction is approx. $k_D = 10^{-8}$, that of the antibody reaction is only $k_D = 10^{-5}$.

It is possible, so to speak by reversing the above method, that explosive molecules are immobilized at the solid phase (e.g. in a light-conducting fiber arranged in a flow cell with connected detector for the light transmitted by a fluorophore upon excitation by means of for instance a light-emitting diode), and a signal is generated by means of the marked nucleic acid. This reversal cannot only serve for an actual measurement (the amount of nucleic acid bound to the solid phase decreases according to the balance, if there are explosive molecules present in the solid or gas phase), but calibration

curves can also be established, or nucleic acids can be tested for their suitability according to the invention.

Nucleic acid or aptamer sequences being suitable according to the invention are given in Fig. 8a to 8h and in Fig. 9. Marked sections (underlined partial sequences) or consensus sections (respectively individual or connected by an arbitrary number of nucleotides) have an independent importance, respectively. Fig. 9 shows variations of the aptamer-consensus sequences; the exchange possibilities mentioned in the columns are provided for nucleotides. The above sequences are also represented in the sequence protocols.

In principle, sequences or partial sequences according to the invention can be used as individual molecules or individually within a larger molecule. They can however be combined in an arbitrary manner with each other in an individual molecule. By means of different sequences according to the invention in a (nucleic acid) molecule, different explosives or a specific explosive can be detected with increased specificity. In the first case, the joined-together sequences are specific for partial molecular structures or overall structures of different explosive molecules. In the latter case, the joined-together sequences are specific for different partial molecular structures of a single explosive molecule ("bi-specific" or "multi-specific"). It is however also possible that identical sequences or partial sequences according to the invention are joined together, so that a single nucleic acid molecule can bind several (identical) explosive molecules. In any case, it

may be recommendable to interpose suitable spacer sequences between sequences or partial sequences according to the invention. Such spacer sequences can be calculated in particular in the case of bi-specific or multi-specific nucleic acids for instance by way of nuclear modeling, and the three-dimensional binding information necessary therefor with regard to the various sequences can for instance be obtained by 3D correlation NMR (e.g. 1H/1H or 1H/14C).